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Purification and treatment of recombinant human RyR2

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An abbreviated version of this protocol was published in Science Advances in Jul 2022

Structural analyses of human ryanodine receptor type 2 channels reveal the mechanisms for sudden cardiac death and treatment

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Detailed protocol

Homogenization buffer (H) 100 mL:
10 mM TRIS malate pH 6.8 (2 mL 0.5 M), 1 mM EGTA (200 uL 0.5M), DTT 1 mM (100 µL 1 M or 15.4 mg), 1 mM Benzamidine (18 mg), 0.5 mM AEBSF (12 mg), 2 protease inhibitor (PI) tablet. Add GST-Cal2.

Buffer S 15 mL:
10 mM HEPES pH 7.4 (300 µL 0.5 M), 0.85 M NaCl (0.75 g), 1.5% CHAPS (225 mg), 0.5% PC (75µL, stock: 10% in 10% CHAPS), 1 mM EGTA (30µL 0.5M), 2 mM DTT (30 µL 1 M or 4.63 mg), 0.5 mM AEBSF (6 mg), 1 mM Benzamidine (8 mg), 1 protease inhibitor (PI) mini tablet. Add GST-Cal2.

Buffer D 50 mL:
10 mM HEPES pH 7.4 (1000 µL 0.5 M), 1.0% CHAPS (500 mg), 0.1% PC (0.5 mL, stock: 10% in 10% CHAPS), 1 mM EGTA (100 µL 0.5M), 2 mM DTT (1 µL 1 M), 1 protease tablet.

Buffer Wa 500 mL:
10 mM HEPES pH 7.4 (10 mL 0.5 M), 0.4% CHAPS (2 g), 1 mM EGTA (1 mL 0.5M), 0.5 mM TCEP (65 mg), 0.001% DOPC (evaporate 0. 2 mL fro chloroform solvent in glass tube and dissolve in buffer W), 230 mM NaCl (6.7 g).

Buffer Wb (100 mL Wa):
NaCl 666 mM (+2.5 g).

Buffer Ge (100mL Wa):
GSH 10mM (0.3g), DTT 1mM (0.1mL 1M), check pH 7.7-8.0.

Protocol:

1. Resuspend cell pellet in buffer H, approx: 1.5 mL/dish. Add GST-Cal2 (40 nmoles, 100 equivalents of 1mg of RyR2). Incubate 15 min. Lysate via sonication (50%, pulses: 2 min; 15 s on, 20 s off).
2. Centrifuge to separate DNA and debris: 10' 3000 xg.
3. Centrifuge to separate membranes: 30' 100.000 xg (30k rpm Ti-45, 34k rpm Ti-55 or Ti-70).
4. Resuspend pellet in 10-15 ml S, adding GST-Cal2 (40 nmoles). Homogenize.
5. Add 35-50 ml D with glass homogenizer. Incubate for 30-60 min in overhead shaker (cold room).
6. Centrifuge to separate insoluble debris: 30' 100.000 xg.
7. Vacuum filter and load onto Q column (5ml), pre-equilibrated with Wa, at 1 mL/min
8. Wash with Wa for 5 CV
9. Elute 3 volumes with isocratic buffer at 40 mS/cm (60-70% buffer Wb) and pool RyR2 fractions.
10. Add 80 nmoles of GST-Cal2.
11. Load into GSTrap column, pre-equilibrated with Wa, at 0.2-0.5 mL/min. Leave ON recirculating.
12. Wash with Wa_{200} for 5-10 CV, at 1-2 mL/min. Attach Q column (1ml). Wash with Wa_{200} for 5 CV
13. Elute with Ge for 5-10 CV or until stabilization of UV. Wash with Wa.
14. Elute with a linear gradient between 30-45 mS/cm NaCl (40-70% buffer Wb. Pool RyR2 fractions.
15. Thrombin digestion: add 50 U of Thrombin. PKA phosohorylation: 100 U PKA, 10 mM EGTA (60 uL 0.5 M), 8 mM MgCl2 (24 ul 1 M), 100 uM ATP (1.6 u 200 mM). Dephosphorylation: 6-12 uL phosphatase lambda, buffer 10X, Mn 10X. Incubate 30 min at RT.
16. Concentrate and load into SEC (TSKgel). Pool RyR2 fractions.
17. Concentrate to 20 uL and filter (centrifugal filters 0.22um).
18. Measure concentration by NanoDrop.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Miotto, M. and Marks, A. (2023). Purification and treatment of recombinant human RyR2. Bio-protocol Preprint. bio-protocol.org/prep2254.
2. Miotto, M. C., Weninger, G., Dridi, H., Yuan, Q., Liu, Y., Wronska, A., Melville, Z., Sittenfeld, L., Reiken, S. and Marks, A. R.(2022). Structural analyses of human ryanodine receptor type 2 channels reveal the mechanisms for sudden cardiac death and treatment. Science Advances 8(29). DOI: 10.1126/sciadv.abo1272